Disaster Medicine and Public Health Preparedness

www.cambridge.org/dmp

Original Research

Cite this article: Wilkins R, Ainsbury EA, Alsbeih GA, Balajee A, Blakely WF, Deminge M, Endesfelder D, Fenech M, Gregoire E, Herrold J, Marrale M, Martinez JS, Miura T, Moreno Domene M, Oestreicher U, Pecoskie S, Prieto MJ, Regulla D, Romanyukha A, Sommer S, Suto Y, Swartz HM, Terzoudi G, Toyoda S and Trompier F (2025). Activities of ISO Working Group 18: Biological and Physical Retrospective Dosimetry. *Disaster Medicine and Public Health Preparedness*, **19**, e251, 1–11
https://doi.org/10.1017/dmp.2025.10095

Received: 11 December 2024
Revised: 11 December 2024
Accepted: 05 February 2025

Keywords:
biodosimetry; EPR; radiation; Standardization; Cytogenetics

Corresponding author:
Ruth Wilkins;
Email: ruth.wilkins@hc-sc.gc.ca

Activities of ISO Working Group 18: Biological and Physical Retrospective Dosimetry

Ruth Wilkins[1], Elizabeth A. Ainsbury[2], Ghazi A. Alsbeih[3], Adayabalam Balajee[4], William F. Blakely[5], Mayra Deminge[6], David Endesfelder[7], Michael Fenech[8], Eric Gregoire[9], Jim Herrold[10], Maurizio Marrale[11], Juan S. Martinez[9], Tomisato Miura[12], Mercedes Moreno Domene[13], Ursula Oestreicher[7], Stephen Pecoskie[14], Maria Jesus Prieto[13], Deiter Regulla[15], Alexander Romanyukha[5], Sylwester Sommer[16], Yumiko Suto[17], Harold M. Swartz[18], Georgia Terzoudi[19], Shin Toyoda[20] and François Trompier[9]

[1]Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, Canada; [2]UK Health Security Agency, Radiation, Chemicals and Environmental Hazards Directorate, Chilton, Oxfordshire, United Kingdom; [3]Biomedical Physics Department, Radiation Biology Section, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; [4]Cytogenetic Biodosimetry Laboratory, Radiation Emergency Assistance Center/Training Site, Oak Ridge Institute for Science and Education, Oak Ridge Associated Universities, Oak Ridge, TN, USA; [5]Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [6]Biological Dosimetry Laboratory, Nuclear Regulatory Authority (ARN), Buenos Aires, Argentina; [7]Federal Office for Radiation Protection, BfS, Oberschleissheim, Germany; [8]Health and Biomedical Innovation, UniSA Clinical and Health Sciences, University of South Australia, Adelaide, Australia; [9]Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PSE-SANTE/SERAMED/LRAcc, F-92260, Fontenay-aux-Roses, France; [10]Safety Office, University of Wyoming, Laramie, WY, USA; [11]Department of Physics and Chemistry "Emilio Segrè," University of Palermo, Palermo, Italy; [12]Department of Risk Analysis and Biodosimetry, Institute of Radiation Emergency Medicine, Hirosaki University, Hirosaki, Japan; [13]Hospital General Universitario Gregorio Marañón; Servicio de Oncología Radioterápica; Laboratorio de dosimetría biológica, Madrid, Spain; [14]Canadian Nuclear Laboratories, Chalk River, Canada; [15]Institute for Clinical Radiology, University of Munich, Munich, Germany; [16]Institute of Nuclear Chemistry and Technology, Warsaw, Poland; [17]National Institutes for Quantum Science and Technology, Chiba, Japan; [18]Department of Radiology, Geisel School of Medicine at Dartmouth, Hanover, NH, USA; [19]National Centre for Scientific Research "Demokritos," Health Physics, Radiobiology & Cytogenetics Laboratory, Agia Paraskevi, Greece and [20]Institute of Palaeontology and Geochronology, Okayama University of Science, 1-1 Ridai, Kita-ku, Okayama, Japan

Abstract

Biological and physical retrospective dosimetry for ionizing radiation exposure is a rapidly growing field, and several methods for performing biological and physical retrospective dosimetry have been developed to provide absorbed dose estimates for individuals after occupational, accidental, intentional, and incidental exposures to ionizing radiation. In large-scale radiological/nuclear incidents, multiple retrospective dosimetry laboratories from several countries may be involved in providing timely dose estimates for effective medical management of several thousand exposed individuals. In such scenarios, the harmonization of methods among participating laboratories is crucial for consistency in data analysis, dose estimation, and medical decision-making. In this regard, ISO documents ensure that these practices are standardized globally across the laboratories by providing quality assurance and quality control documentation that guide laboratories in maintaining high-quality performance for consistency. With the intent of bringing standardization and harmonization of biological and physical retrospective dosimetry methodologies across national and international laboratories, the ISO working group 18 (WG18) was established under ISO/TC85/SC2 (Technical Committee 85, Subcommittee 2-Radiation Protection) in 1999. This manuscript summarizes some of the past, current, and future activities of WG18 on biological and physical retrospective dosimetry.

Introduction

Importance of Standards in Life Sciences and Industries

The International Organization for Standardization (ISO) facilitates the harmonization of operating procedures, such as laboratory protocols, to ensure that results are comparable and reproducible across different laboratories when the same methods are used. This harmonization of procedures is crucial for maintaining uniformity, reliability, consistency, and quality in industrial, medical, and educational operations. By providing guidelines and procedures, ISO standards not only help the quality control (QC) and quality assurance (QA) assessments but also improve the productivity of the organizations and industries by identifying and minimizing

inefficiencies through implementation of corrective action plans. ISO documents are available for the management of industries, environment, health and safety, and food safety, among others, and these documents are developed and established by the scientific wisdom of subject matter experts working as a group. There are almost 300 technical committees that are currently involved in developing and establishing ISO standards for different disciplines.

The standards established by ISO have been in routine use in the industrial world for several decades and have found their way into the biological sciences, where standards are crucial for reliability of procedures, quality assurance, data comparison, and data validation. Radiation biological and physical retrospective dosimetry is an actively emerging field of science where several tools have been and continue to be developed for estimating the absorbed radiation dose in potentially exposed humans. ISO standards are needed for each of the biological and physical retrospective dosimetry assays to establish consensus methodologies for dose estimation either by individual or national/international dosimetry network laboratories, specifically for use in the aftermath of mass-casualty radiological or nuclear incidents/accidents. In such a scenario, ISO standards for harmonizing the protocols among the laboratories are imperative to get consistent and reliable results for fair data comparison and validation of dose estimates.[1] Additionally, these ISO standards may be useful for accreditation of newly developed retrospective dosimetry laboratories. Realizing the importance of ISO standards for these laboratories, a technical committee (ISO/TC85/SC2) working group (WG18) was established in 1999 to develop standards for some of the established biological dosimetry tools. This was later expanded to include retrospective physical dosimetry through the establishment of a subworking group focused on electron paramagnetic resonance (EPR). This manuscript summarizes some of the past, current, and future activities of the WG18 on biological and physical retrospective dosimetry standards.

Background on biological and physical retrospective dosimetry

Biological and physical retrospective dosimetry is the measurement of absorbed radiation dose based on the response of biological indicators such as DNA, RNA, proteins, lipids as well as a variety of metabolic byproducts or physical measurements in biological material such as EPR in teeth or bones. Retrospective dosimetry can be used to estimate the absorbed radiation dose when individuals without personal dosimeters are exposed to unknown doses of ionizing radiation. It can also be used to confirm the radiation dose on a personal dosimeter in the case of radiation/nuclear workers and to gain information, in some instances, about the nature and level of exposures. Biological dosimetry was developed in the mid-20th century as it became evident that chromosomal aberrations increased in a dose-responsive manner after exposure to ionizing radiation and could be used as an indicator of dose to the exposed individual. The use of biological dosimetry after the "Recuplex" criticality accident in Hanford, USA,[2] and after a radiation accident in 1969 involving industrial radiographers in the UK[3] is an early example of its application. Throughout the second half of the 20th century, biological dosimetry was used for diverse nuclear accidents and incidents, which allowed for not only the dose assessment but also the improvements of the existing techniques.[4–9]

In the case of large-scale radiological/nuclear incidents/accidents, several hundreds and thousands of people could be exposed to substantial doses of ionizing radiation. The $LD_{50/60}$ (the lethal dose that will kill 50% of the exposed individuals in 60 days) value ranges from 3.5 Gy to 4 Gy without supportive care and more than 7 Gy with supportive care. Therefore, timely assessment of absorbed radiation dose will be useful for saving human lives.[10] Figure 1 summarizes the methods that can be used, alone or in combination, to perform radiation dose estimates in these scenarios.

Overview of ISO and WG18

Life Cycle of Developing ISO Standards

The ISO standards in most cases contain tested and validated procedures or protocols for the best practices in any business, industrial, and educational/research organizations. There are 2 basic steps for the development of standards: (1) consensus among the subject matter experts about the added value an ISO standard would bring in a specific area and (2) acceptance of a standard at the national level.

Once there is consensus among experts in the field that a new standard would be of value, a proposal is then submitted to the relevant ISO technical committee composed of representatives from various countries and stakeholder groups. Bringing an ISO standard to life is a well-defined process, with each stage acting as a crucial checkpoint toward publication. While the timeframe might vary (18, 24, or 36 months), the core stages remain essential for every standard, ensuring thoroughness, consensus, and global relevance. The life cycle of an ISO standard is depicted in Figure 2.

The rigorous review process is the foundation of ISO standards' credibility and trust. By incorporating diverse perspectives and expertise, the standards reflect the global needs of the field they serve. Subsequent sections of this manuscript will delve into the specific applications of ISO standards in retrospective dosimetry, which is a specialty under the working group ISO/TC 85/SC 2/WG18: biological and physical retrospective dosimetry.

History of Identifying the Need for Standardized Biological and Physical Retrospective Dosimetry Techniques

In the 1990s, there were several accidental exposures to ionizing radiation that had no accompanying physical dosimetry and, at that time, only a few biological dosimetry laboratories were available for conducting the analysis. Although biological dosimetry was available, there were issues with the acceptance of the results, particularly if they were not as expected. One solution to address the lack of confidence in the results was to establish an international intercomparison program. The first of these was conducted in 1995 with 2 laboratories, the Nuclear Safety and Protection Institute (IPSN, now Institut de Radioprotection et de Sûreté Nucléaire, IRSN, France) and what was then known as the National Radiological Protection Board (NRPB, now UK Health Security Agency, UKHSA). A second intercomparison was held in 2002 with 15 laboratories and included exposure to both neutrons and gamma rays.[11] Even though the estimations from 11 of the 15 participants fell within the ±30% of the physical reference dose, the results indicated some discrepancies in the rate of dicentrics enumerated among participants. As fixed cells were transported to each laboratory, 1 explanation was that the quality of metaphases was affected by the transportation of the samples. Additionally, slide-making procedures may have varied between laboratories. The differences in dicentric scoring among participants could be also due to the type of radiation used and inexperience at scoring highly damaged cells. With high-LET radiations, some metaphases may have contained numerous chromosomal aberrations such as multicentric

https://doi.org/10.1017/dmp.2025.10095 Published online by Cambridge University Press

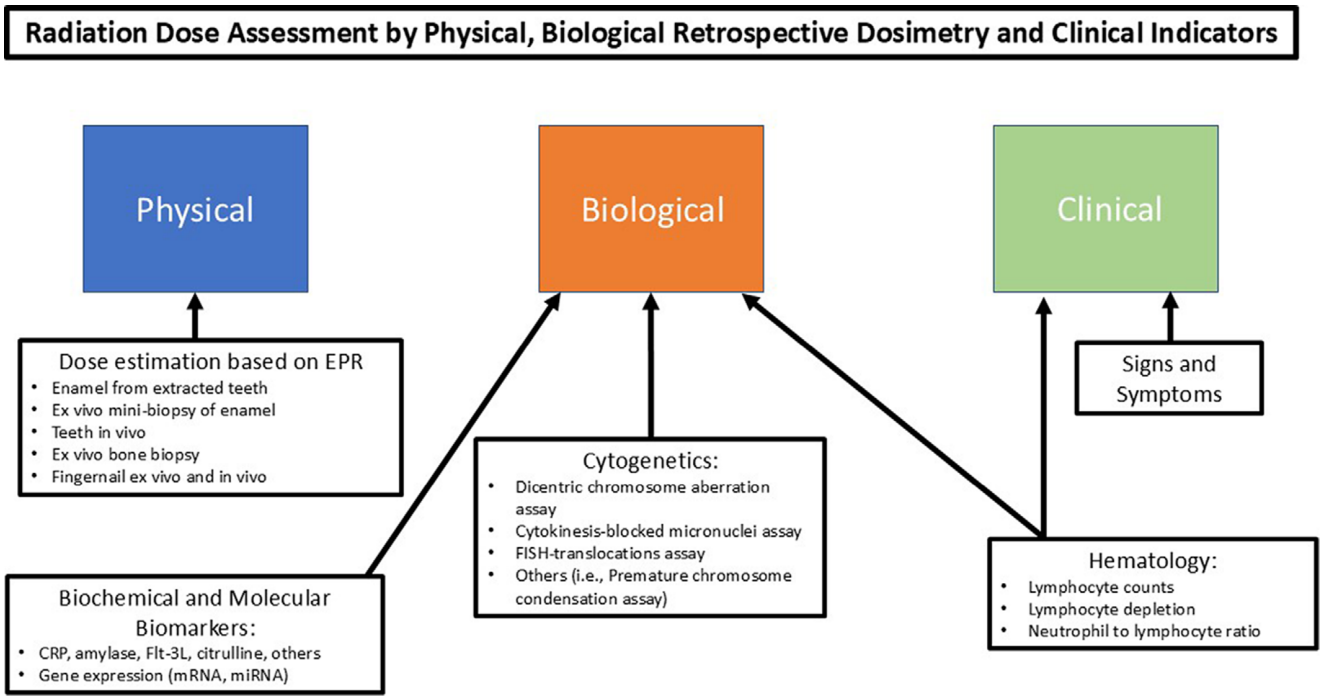

Figure 1. Representation of different physical, biophysical, biological, and clinical dosimetry methods for radiation dose assessment.

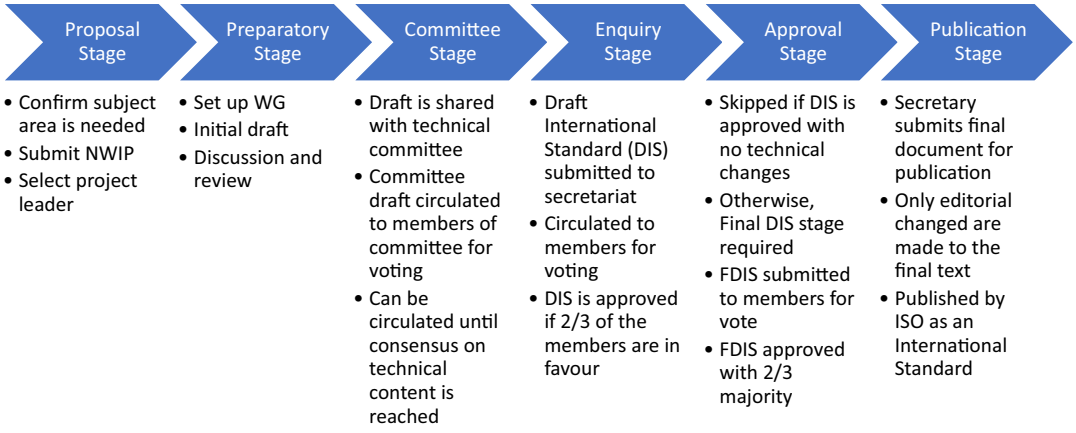

Figure 2. Depiction of the stages of an ISO document from proposal through to publication.

chromosomes and centric rings which can make the scoring difficult. Another issue with the dose estimates could have been the dose-response curve used. Few of the participant laboratories had a neutron dose response curve, while others used their gamma or X-ray dose-response curves. However, smaller variations were observed in the dose estimations as compared to the frequency of aberrations, highlighting that experimental conditions such as slide making and scoring can be laboratory dependent.

A second solution to address the lack of confidence was to establish a process for laboratory accreditation by an independent international body.[12] At that time, there were only 2 applicable standards for laboratories:

- 9001:1987—Quality systems—model for quality assurance in design/development, production, installation and servicing (now 9001:2015 Quality management systems—requirements)[13]
- ISO/IEC Guide 25:1990 General requirements for the competence of calibration and testing laboratories (now 17025:2017)[14]

It was clear that there was a need to create standards specific to biological dosimetry techniques. In 1999 the ISO working group 18 "Biological Dosimetry" was formed under Technical Committee 85 (Nuclear energy, nuclear technologies, and radiation protection)/Subcommittee 2 (Radiological Protection). The WG consisted of 13 specialists from 11 countries who met for the first time at IRSN and started drafting the first standard on the dicentric assay.

Since its conception, the WG has expanded to include physical retrospective dosimetry and in 2021 changed its name to "Biological and physical retrospective dosimetry" reflecting the addition of standards on EPR. The committee has now developed a suite of standards including guidance on conducting the dicentric assay, the

https://doi.org/10.1017/dmp.2025.10095 Published online by Cambridge University Press

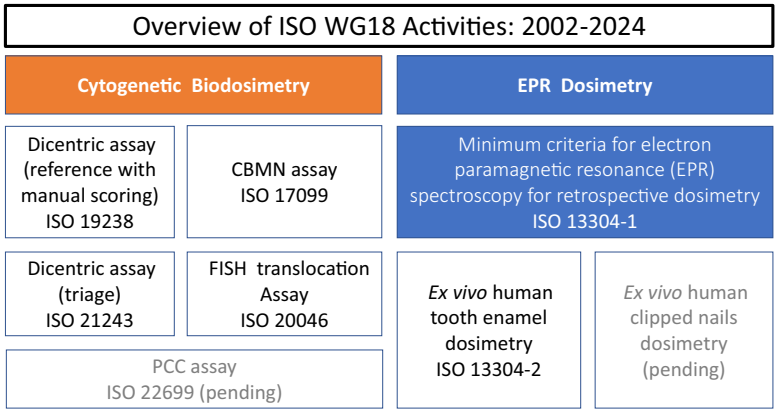

Figure 3. Overview of activities of ISO WG18.

cytokinesis-block micronucleus assay, translocation analysis using FISH and EPR (Figure 3). A list of these standards can be found in Table 1.

A typical standard for a specific assay will contain detailed technical information on various procedural steps, starting from sample collection, followed by assay procedure, data generation, data analysis, dose estimation, and report generation. A detailed description of statistical analysis for dose estimation will also be included in the standard. It will also contain details on quality assurance and quality control procedures specific to the assay. Often examples of useful documents are included in the Annexes (e.g., instructions for requestors, questionnaires, reporting templates).

Table 1. List of standards published by WG18

ISO #	Standard title	Date first published	Date of latest revision
19238	Radiation protection -Performance criteria for service laboratories performing biological dosimetry by cytogenetics	2004	2023
21243	Radiation Protection- Performance criteria for laboratories performing initial cytogenetic dose assessment of mass casualties in radiological or nuclear emergencies — General principles and application to dicentric assay	2008	2022
17099	Radiological protection - Performance criteria for laboratories using the cytokinesis block micronucleus (CBMN) assay in peripheral blood lymphocytes for biological dosimetry	2014	2024
20046	Radiological protection — Performance criteria for laboratories using Fluorescence In Situ Hybridization (FISH) translocation assay for assessment of exposure to ionizing radiation	2019	2019
13304–1	Radiological protection — Minimum criteria for electron paramagnetic resonance (EPR) spectroscopy for retrospective dosimetry of ionizing radiation — Part 1: General principles	2013	2020
13304–2	Radiological protection — Minimum criteria for electron paramagnetic resonance (EPR) spectroscopy for retrospective dosimetry of ionizing radiation — Part 2: ex vivo human tooth enamel dosimetry	2020	2020

ISO Standards for Biological and Physical Retrospective Dosimetry on Biological Samples

Ionizing radiation (IR) induces a wide spectrum of lesions on chromosomal DNA. DNA double-strand breaks are critical lesions which, when mis-rejoined, can result in microscopically detectable structural chromosome alterations. For this reason, various cytogenetic methods for detecting the structural chromosome aberrations have been developed and routinely used for radiation cytogenetic biological dosimetry. Methods for retrospective analysis using physical dosimetry have also been developed. Table 2 lists the biological and physical retrospective dosimetry assays that have ISO standards or are under consideration for standardization. A brief account of these assays is provided below, with the associated standards listed in Table 1:

Dicentric chromosome assay (DCA)[15,16]

The DCA is a cytogenetic assay that is considered the gold standard of biological dosimetry and is the most used method.[1,4] This assay is performed either on whole peripheral blood cells or on isolated lymphocyte cultures and involves the scoring of dicentric chromosomes that are formed after the mis-rejoining of 2 broken chromosomes with intact centromeres and can be easily visualized in metaphase cells (Figure 4A). Identification of dicentric chromosomes can also be optimized by the addition of peptide nucleic acid (PNA) probe specific for centromeres of all the human chromosomes. Dicentric-bearing lymphocytes are unstable when passing through mitosis; therefore, they disappear with time.[17,18] Their persistence in the human body is estimated to be between 6 and 12 months after exposure[19] and therefore DCA is most suitable for acute recent exposures. Since *in vitro* culturing of lymphocytes for 48 h is required for chromosome preparation, the turnaround time for DCA-based dose estimation is usually 72-96 h. The DCA has been used in many instances of dose reconstruction for both small and large numbers of exposed individuals.[4–9]

In the case of a radiological emergency, triage methods shorten the analysis time and provide timely information to the medical

https://doi.org/10.1017/dmp.2025.10095 Published online by Cambridge University Press

Table 2. Comparison of assays used for dose assessment (modified from the IAEA cytogenetic manual.[4])

Assays	Endpoints	Radiation scenarios			Acute photon dose range, Gy	Dose applications		ISO standard
		Acute	Protracted	Prior[†]		Partial-body	Triage	
Dicentric chromosome assay (DCA)	Dicentrics (and rings)	Yes	Yes	Not ideal	0.1–6	Yes	Yes	Yes
Cytokinesis-blocked micronucleus (CBMN)	Micronuclei in binucleated cells, nucleoplasmic bridges	Yes	Yes	Not ideal	0.3–5	No	Yes	Yes
Fluorescence in situ hybridization (FISH)	Dicentrics (and rings), translocations	Yes	Yes	Yes	0.25–5	No	No	Yes
Premature chromosome condensation (PCC)[#]	Excess fragments, rings, dicentrics, and length ratio	Yes	Yes	Not ideal	~0.2–30 (depending on endpoint)	Yes	Yes	Pending
Electron paramagnetic resonance (EPR)	EPR signal from nail clippings, intact nails (*in vivo*). Tooth (*in vivo*), or tooth enamel biopsies, tooth enamel extracted teeth	Yes	Yes	Yes	0.05 (tooth enamel, extracted teeth) >0.4 (enamel biopsies) >2 (nail clippings, nails *in vivo*, teeth *in vivo*)	Yes	Yes	Yes (for *ex vivo* tooth)

[†]Prior: An assessment of dose when blood sampling is performed greater than 3 months after radiation exposure.
[#]With or without the use of centromeric and/or whole-chromosome specific hybridization probes.

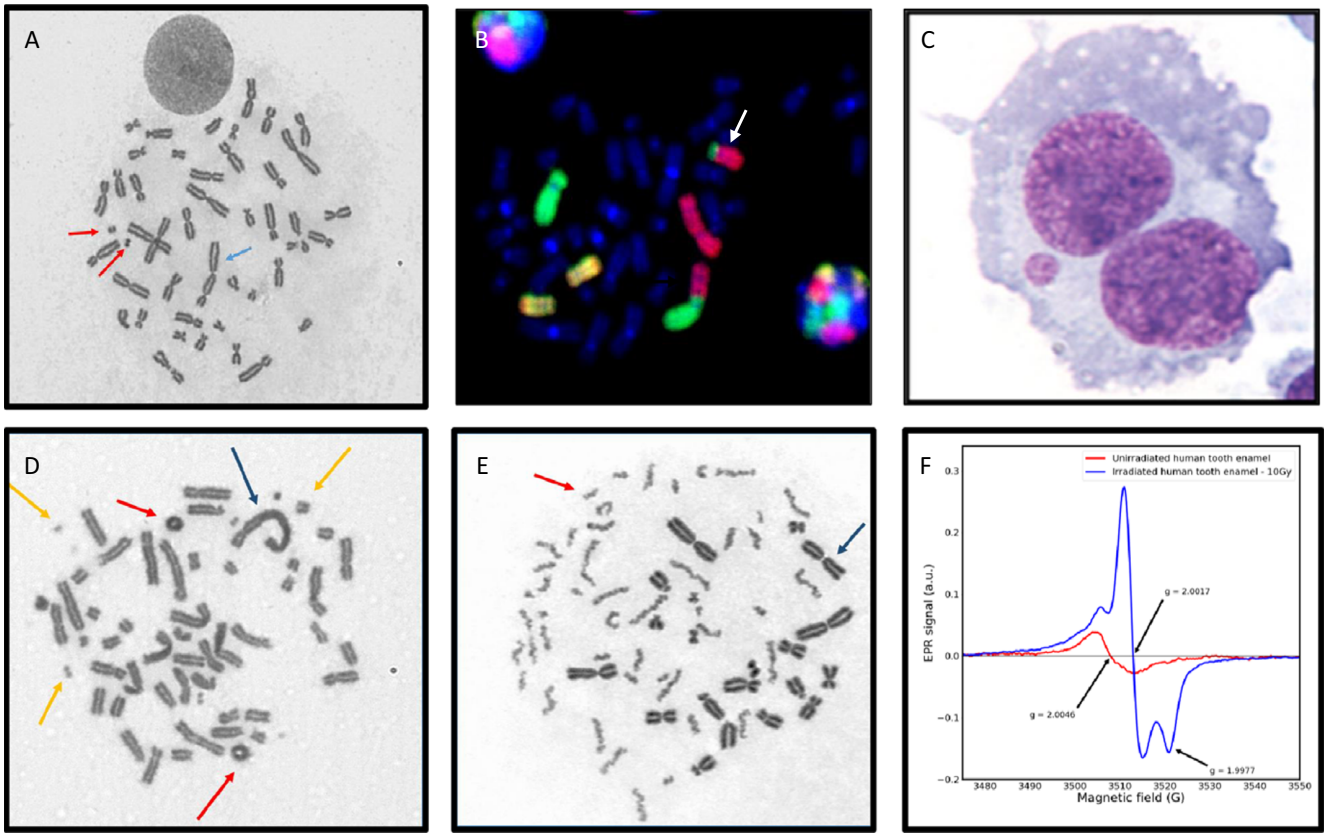

Figure 4. Representative images illustrating damage detection by some of the commonly used biological dosimetry assays. (A) Metaphase spread from the dicentric chromosome assay showing a dicentric (blue arrow) and 2 acentric fragments (red arrows). (B) A symmetrical translocation (white arrow) detected by FISH using a cocktail of fluorescently labeled chromosome-specific DNA probes. (C) A binucleated cell with 1 micronuclei detected by the cytokinesis-block micronucleus assay. (D) A G2-phase cell after chemically induced PCC assay showing a dicentric (blue arrow), 2 rings (red arrows), and 3 acentric fragments (yellow arrows). (E) PCC chromosome (red arrow) along with metaphase CHO chromosomes (blue arrow) from the PCC cell fusion assay and (F) 2 EPR spectra of dental enamel (irradiated and non-irradiated) and included g-values.

https://doi.org/10.1017/dmp.2025.10095 Published online by Cambridge University Press

team to assist in the care of potentially exposed victims. In cytogenetics-based biological dosimetry, triage analysis includes manually scoring either 50 metaphases or 30 dicentrics (whichever is reached first), allowing dose estimates within ±1 Gy of the absorbed dose.[4] This margin of uncertainty is sufficient to guide the medical teams in the initial treatment. Another triage option is to conduct semiautomatic analysis of 500 to 1000 metaphases, which could potentially shorten the analysis time.[5]

Translocation analysis[20]

Similar to the DCA, translocation analysis is performed on mitotic lymphocytes; however, the translocations, which involve the exchange of parts of the chromosomes between 2 or more chromosomes, are detected by fluorescence *in situ* hybridization (FISH) using probes for a specific set of chromosomes or the whole complement (Figure 4B). Symmetrical translocations, in which each resultant chromosome has 1 centromere, are considered stable aberrations that can pass through mitosis (unlike dicentric chromosomes) and are more easily transmitted to progeny cells. This allows them to persist in the body for many years (much longer than dicentric chromosomes) and hence are useful for retrospective biological dosimetry several years after radiation exposure. These translocations, however, increase with age and other lifestyle factors and should be corrected for when calculating dose estimates.[21] Some notable examples of cases where FISH-based biological dosimetry was employed include the follow-up of victims from the Hiroshima-Nagasaki bombings[22]; children living close to the Chernobyl nuclear accident site[23,24]; victims of the Goiânia accident[25,26]; victims of the Tallin incident[27,28]; and the workers from the Mayak nuclear weapon manufacturing site in the Techa river region (Russia), which was contaminated mainly by radioactive strontium in the 1950s.[29]

Cytokinesis-block micronucleus (CBMN) assay[30]

In the lymphocyte cytokinesis-block micronucleus assay, micronuclei resulting from mal-segregation of whole chromosomes or chromosome fragments that are excluded from the main nuclei are scored *ex vivo* in cytokinesis-blocked binucleate cells (Figure 4C).[4] Unlike dicentric chromosomes which are specific for radiation exposure, micronuclei can be induced by a variety of genotoxic agents including ionizing radiation. The CBMN assay has been applied to several accidental scenarios for retrospective dose reconstruction.[7,31–33]

Premature chromosome condensation (PCC) assay

This assay involves the use of protein kinase inhibitors (calyculin A and okadaic acid) to prematurely condense the chromosomes in the G2 phase of the cell cycle prior to reaching mitosis. This allows the visualization of chromosomes in heavily damaged cells that are permanently arrested in the G2-phase and are unable to progress to mitosis (Figure 4D).[34] Alternatively, G0-PCC can be induced where the irradiated cells (usually G0-human lymphocytes) are fused with mitotic cells of Chinese Hamster Ovary using polyethylene glycol (PEG) as fusogen (Figure 4E).[35] G0-PCC is advantageous because it can be performed directly after blood collection without the need for lymphocyte culturing as in the case of G2-PCC. One of the great advantages of the PCC assay is its capability to detect higher doses of exposure, thus being complementary to the DCA. In addition, its shorter culture time allows for a rapid dose estimation (few hours). It was notably used for victims of the Tokai-Mura criticality accident, where 2 individuals were exposed to doses higher than 6 Gy[36,37] and in the Taiyuan radionuclide manufacturing accident of 2008 where it was concluded that 5 employees exposed to gamma irradiation received doses ranging from 2 to 12 Gy.[38] This method is being proposed for the next standard to be drafted in WG18.

Electron paramagnetic resonance (EPR) dosimetry[39,40]

Electron paramagnetic resonance (EPR) dosimetry can be defined as a method of radiation dose determination based on the measurements of concentration of radiation-induced radicals in matter with EPR spectroscopy. EPR dosimetry is used to measure radiation doses in solid tissues of humans or animals (teeth *in vitro* and *in vivo*, bone *in vitro*, finger- and toenails *in vitro* and *in vivo*) but also personal items such as cell phone screens, watch glasses, clothes, etc. (not addressed in the framework of WG18). This method has the advantage of being non-destructive to the material being measured, so that repeated measurements on the same sample can be made. The other attractive features of the EPR dosimetry include linear dose dependence for most materials and tissues, the possibility to do dose measurements immediately after irradiation, based on a physical process that is not affected by biological processes such as stress or simultaneous insults/damage that are likely to occur with irradiation such as wounds and burns. The timeframe for measurements can be made at any interval immediately after irradiation up to at least 2 weeks (fingernails), indefinitely (teeth),[41] or a range of times for other EPR dosimetry materials. Furthermore, the EPR signal is unaffected by dose rate and can be used for dose measurements after partial body exposures.

ISO 13304-1:2020 provides a general guidance on sample collection, their preparation for the dose measurements, EPR measurements in the samples under investigation, calibration of the EPR radiation response in the radiation dose units, and some other useful information related to the application of this technique. This standard covers *in vitro* and *in vivo* EPR dose measurements in biological tissues (mostly teeth and nails) of the person, personal items, and objects located in the immediate environment to the human. It usually employs X-band frequency (8-12 GHz), but higher and lower frequencies are also being considered. Specifically, this International Standard proposes a methodological frame and recommendations to set up, validate, and apply protocols from sample collection to dose reporting. However, this standard does not provide any specificity to the type of the sample (e.g., teeth, nails, and other applicable materials) used for the dose measurements. ICRU reports 68 and 94 give an analysis of the most commonly available methods of retrospective dosimetry, including EPR.[42,43] These 2 reports provide extensive lists of the references on the methodology and applications of EPR dosimetry.

Standard ISO 13304-2:2020 provides specific guidelines to perform the *ex vivo* measurements of human tooth enamel samples by X-band EPR for dose assessment (Figure 4F). This technique is the most developed EPR dosimetry method, which has been applied to reconstruct radiation doses received by Hiroshima and Nagasaki A-bombardment survivors, population and emergency personnel affected by the Chernobyl accident, Southern Ural population in Russia residing in the areas contaminated by the Russian nuclear weapon plant Mayak, and in many other radio-epidemiological studies.[42,44] ISO 13304-2:2020 expands and standardizes the measurement and dose reconstruction procedures and the evaluation of performance. This document is compliant with ISO 13304-1 "Radiological protection—minimum criteria for electron paramagnetic resonance (EPR) spectroscopy for retrospective dosimetry of ionizing radiation—Part 1: General principles" with particular

https://doi.org/10.1017/dmp.2025.10095 Published online by Cambridge University Press

consideration given to the specific needs of X-band EPR dosimetry using human tooth enamel.

In the context of radiological accidents, this method has been mostly used on biopsies of bone tissues, providing very pertinent information when irradiation is localized to a small volume of the body and to a less extent to biopsies of tooth enamel.[43] *Ex vivo* tooth enamel dosimetry (on whole tooth) has been largely used in support of epidemiological studies of ancient and/or prolonged exposure.[42]

Factors involved in radiation dose assessment

There are many aspects of radiation dose assessment by biological or physical retrospective dosimetry that are addressed within the standards:

(1) Radiation exposure parameters can vary from one exposure scenario to the next: level of dose, radiation quality, dose rate including single or fractionated doses, and whether a person is entirely or partially exposed. Each of these factors will affect the dose estimates made and these are addressed in the standards. The amount of radiation one is exposed to can determine the number of cells required to be analyzed for the cytogenetic assays. Different qualities of radiation affect the shape of the dose response curves and advice is provided on the range and number of doses required when creating a dose response curve for high- and low-LET radiation. The dose rate also affects the shape of the dose response and advice is provided on how to apply an acute dose-response curve to non-acute exposures. Finally, it is of great importance to understand whether an individual has been exposed to a whole- or partial-body exposure, as the medical intervention will differ in these 2 cases. The standards describe methods to determine whether an exposure is partial body.

(2) There are continuous improvements to the assays that must reach a certain level of maturity and uptake before being incorporated into the standards. These are assessed each time a standard comes up for review. One example is the development of software tools for performing data and statistical analysis for cytogenetic assays. Although specific software is not mentioned, they are mentioned as an acceptable tool. Automation is another improvement that is becoming widely used but has not yet been described in detail in a standard.

(3) The type of dose assessment methodology to be employed may depend on the exposure scenario. For early response situations, the DCA, CBMN, or EPR standards might be followed, whereas for a retrospective analysis, at longer times postexposure, translocation analysis, or EPR would be applicable (Table 2). Depending on the number of casualties requiring analysis, one might consider employing triage scoring that has been described in ISO 21243,[45] which outlines scoring methodology for rapid throughput and activation of networks for sample sharing to establish surge capacity when a laboratory's capacity has been exceeded. This standard could be applied to any of the other standards.

(4) ISO 21243 also includes guidance on preparedness of a laboratory network prior to an event including organization, harmonization of protocols, quality assurance and control, training and intercomparison exercises to demonstrate capacities, and capabilities of network laboratories to maintain performance criteria and continuous exchange of scientific and technical information.

Use and impact of standards/achievements

Networks

In the case of large-scale accidents, the number of victims can easily exceed the capacity of single laboratories or organizations. Networking between experienced laboratories has been identified as a useful and important strategy in emergency management to overcome this bottleneck by sharing the workload and providing mutual assistance between laboratories. Besides increasing capacity, networking allows the broadening of the spectrum of methods and the choice of the most suitable approach for a particular scenario. In recent years, several laboratories around the world have joined forces and set up regional and/or global networks either on a formal or informal basis.[46] Global networks have been established by the World Health Organization (WHO) (BioDoseNet),[47,48] the Radiation Emergency Medical Preparedness Assistance Network (REMPAN), and the International Atomic Energy Agency (IAEA) Response and Assistance Network (RANET).

On the regional level, the key networks are the European Network for Biological and Retrospective Physical Dosimetry (RENEB), the European Radiation Dosimetry Group (EURADOS), the Latin American Biological Dosimetry Network (LBDNet), the North American Network, and the Asian Radiation Dosimetry Group (ARADOS). The RENEB network of biological and physical retrospective dosimetry laboratories has been operating in Europe since 2017. The network currently includes 16 voting members and 43 associate members from Europe and Asia.[49] The North American network is an informal network established first in Canada in 2002 and has now expanded to include several US laboratories.[50,51] LBDNet was founded in 2007 and originally comprised 7 countries.[52] ARADOS[53] was established in October 2015 by researchers from China, South Korea, and Japan. It consists of a host institute (China, Korea, or Japan, alternately) and 4 working groups (WGs) on Internal Dosimetry, External Dosimetry, Biological Dosimetry, and Computational Dosimetry. In addition, India has also established a national biological dosimetry network involving 7 laboratories.[54]

An essential requirement to achieve comparable results between the laboratory in a network is the harmonization of protocols and standardization of procedures. ISO 21243[45] provides excellent guidance on the aspects of establishing a network, while the ISO standards on individual techniques provide invaluable guidance for harmonizing procedures across laboratories.[55]

Inter-laboratory comparisons

The organization of exercises and interlaboratory comparisons (ILC) are important tools to guarantee high-quality results and to improve and optimize a network's performance. Coordination of infrastructure, logistical aspects, data management, and communication are essential for managing large-scale accidents in biological and physical retrospective dosimetry to increase the throughput and to validate the workflow in a sustainable and operational network. Training and education programs and activities help to improve the ability of network members to provide reliable dose estimates. Furthermore, differences in the experimental setup or the radiation source used by the organizing institutions can help to identify potential issues and to improve and optimize the performance of the network.[49] International ILCs have been mainly run through RENEB,[56–59] EURADOS,[55,60] LBDNet,[52] ARADOS,[53] or Canada.[61] Typically, dose reconstruction is performed using several methods: manual and automatic dicentric chromosome analysis,

https://doi.org/10.1017/dmp.2025.10095 Published online by Cambridge University Press

manual and automatic micronucleus assay, translocation analysis, histone γH2AX foci assay, PCC fusion, gene expression analysis, and EPR. These ILCs are highly dependent on the use of protocols based on ISO standards developed in the frame of WG18, IAEA technical publications,[4] and scientific publications. The focus of the RENEB exercises was variable and included tele-scoring exercises, triage, and full mode scoring for biological dosimetry methods, irradiation with different sources, homogeneous and heterogeneous irradiations, a field exercise, and comparisons between different assays.[55] There have also been several ILCs where performance of the EPR dosimetry in tooth enamel at different laboratories was compared. The most comprehensive one was carried out in 2010.[62] Based on the results obtained by each participant, critical dose and detection limits were calculated by the organizers. The most recent comprehensive RENEB ILC was conducted in 2022[63] in which the performance quality was compared between the established cytogenetic assays, molecular biological assays (γH2AX foci, gene expression), and physical dosimetry-based assays (EPR, optically or thermally stimulated luminescence). The results of all ILCs have contributed to harmonization of SOPs and statistical methods for uncertainty assessment within the RENEB network and EURADOS that have also fed back into revision of ISO standards for biological dosimetry.

Future Perspectives

New Standards Under Consideration

Premature chromosome condensation (PCC) is under consideration for the next biological dosimetry standard. It has been adopted widely by the biological dosimetry community and has advantages over other cytogenetic techniques as described above. A standard dedicated to automation of different aspects of the biological dosimetry methods is also under consideration. This could potentially include both sample processing and scoring of aberrations.

Emerging Assays for Future Consideration

There are several assays that are emerging as biological dosimeters but have not yet reached the maturity for standardization, for example, the γH2AX foci assay. The histone H2AX, a variant of histone H2A, which is a part of the histone octamer on which DNA is wound and is one of the first proteins that responds to DNA damage after radiation exposure through phosphorylation at serine 139 to the γH2AX form.[64] Fluorescent conjugated antibodies are commercially available for easy visualization of the γH2AX foci that form at the sites of DNA double-strand breaks in a radiation dose-dependent manner. Although the histone γH2AX assay is considered a novel biological dosimetric method, with sensitivity in the low dose range (in the order of tens of mSv) and applicability up at least 10 Gy,[65] many technical and methodological problems must still be solved, most notably the fast appearance (optimal peak time is 30 minutes to an hour after exposure) and disappearance of histone foci over time with only about 20% of the initial number of foci remain after 24 h.[66] This means that dose estimation is limited to a short time after the event necessitating the immediate shipment of samples, preferably on ice, to the biological dosimetry laboratories for analysis. Nevertheless, γH2AX assay can still be effectively used as a biological dosimeter if appropriate *in vitro* calibration curves are constructed for different post-exposure times to account for the disappearance of γH2AX foci.

Another emerging technique is measuring changes in gene expression that can be measured in blood and lymphocytes post-exposure to ionizing radiation, showing promise for biological dosimetry and prediction of clinical outcomes.[67–69] Typically, a panel of genes encoding proteins related to the DNA damage response (e.g., DDB2, CDKN1A, GADD45A), apoptosis (e.g., FDXR, BAX, BBC3), and the development of the inflammatory reaction (e.g., GDF15, TNFSF4) is examined, showing significant and repeatable expression changes in peripheral blood cells.[70–72] The research and literature data confirm that the level of transcripts of various genes in the blood shows a clear dependence on the absorbed radiation dose at specified time points after exposure in the range of at least 0.2 Gy to 4 Gy. The method of analyzing gene expression changes is a fast and low-cost method that can be used in the case of a large-scale radiation exposure scenario.

Automation of the DCA and CBMN Assay

One of the main disadvantages of DCA is that manual scoring of dicentric chromosomes is laborious and time-consuming, limiting the sample throughput of a single laboratory that would quickly become overwhelmed in the event of a mass casualty incident. Automation of DCA has been developed over the last 20 years[5,73,74] and has been successfully tested in the framework of the MULTIBIODOSE EU FP7 project.[75,76] To date, several automatic DCA analysis systems have been developed and are in development.[77–81] The use of artificial intelligence (AI) also offers new opportunities to increase capacity in biological dosimetry and to support current and future research in biological dosimetry.[73,82] Automation and semiautomation of the DCA and the CBMN assay have been briefly mentioned in both ISO 19238 and ISO 17099[15,30]; however, due to the rapid development of automation algorithms and use of AI-based tools for automation, these aspects need to be carefully considered by ISO WG18 before incorporating them in the future standards.

Analysis of MN in CBMN is not as technically demanding as the DCA which makes it more amenable to automation, drastically reducing the scoring time. Automatic and semiautomatic MN analysis has been used in many laboratories around the world in recent years.[83–86] Several of these automated CBMN methods are becoming mature enough to be considered for ISO standardization. As this method is also widely used outside of the biological dosimetry community, a standard on automation would be of use in the analysis of cytotoxic and genotoxic effects of environmental mutagens, carcinogens, and clastogens.

Regardless of the automation tool or application, these systems must be validated against the standardized techniques prior to the incorporation into an ISO standard.

Electron Paramagnetic Resonance (EPR) Dosimetry Standardization for Large-Scale Event

In vivo EPR of teeth and finger/toenails offers the advantage of performing onsite measurements of individuals after a small or large-scale radiation event. While its feasibility for triage for such events has been demonstrated, more development/refinement is needed for its acceptance as a standardized technique.[87–89] In addition, *ex vivo* Q-band EPR on tooth enamel mini-biopsies has already been used for an actual radiological accident.[90] As the measurement takes less than 5 minutes after the biopsy is obtained and there are minimal requirements for sample preparation, this method has the potential to be implemented on a large scale, though

https://doi.org/10.1017/dmp.2025.10095 Published online by Cambridge University Press

no organized efforts are in place at this time.[91] Furthermore, AI could help these retrospective EPR dosimetric techniques by automating data analysis, improving calibration accuracy, and facilitating standardization. Indeed, AI algorithms can be trained to automatically identify and quantify EPR signals as well as AI-driven calibration models can improve the accuracy and precision of EPR measurements by accounting for various factors such as sample variability, significantly reducing analysis time.[92] Coordinated efforts could allow for more rapid standardization of technique also in case of adoption of AI tools.

Limitations of Standards

Although standardization of biological and physical dosimetry methods is an essential step in harmonizing laboratories and ensuring robust dose estimates, standards can also have some limitations and downfalls. As the working group is comprised of international experts from different laboratories, there can be difficulties in reaching international consensus on the best procedures when drafting the standards. These differences are usually resolved through thoughtful discussions and compromise, often keeping detailed instructions for crucial points and allowing flexibility where possible so as not to be excessively restrictive.

The ISO standards should be the set of best practices, the foundation for technical and organizational usage of the described method but should also be easily applied. While retrospective dosimetry ISO standards for biological material are often used as a reference because of their scientific credibility, only a few laboratories around the world are accredited against these standards. Therefore, it is also important to ensure that the requirements of the procedures are not so prescriptive that they become too difficult to meet, making accreditation against the standard a challenge. Furthermore, maintenance of experienced personnel and a well-equipped laboratory, as required to comply with the standard, can be resource-intensive.

Conclusions

The ISO WG18: "Biological and Physical Retrospective Dosimetry" has been an active working group since 1999. This WG, comprises members from many countries, has developed and revised 6 standards related to biological and physical retrospective dosimetry, 5 focused on methodology, and 1 on operational processes for large-scale events. These standards have been of great value to the establishment of networks for harmonizing methods across the network laboratories. Future developments in this area will focus on the revision of current standards and on the development of new and emerging techniques as they become sufficiently developed and validated for standardization.

Acknowledgments/Disclaimer. Mention of any brand name products does not imply endorsement. The opinions, conclusions, and recommendations herein expressed are those of the authors and do not necessarily reflect the official policy or position of the Uniformed Services University or the United States Department of Defense. This work was prepared by a civilian employee of the US Government as part of the individual's official duties and therefore is in the public domain and does not possess copyright protection.

References

1. **Voisin P.** Standards in biological dosimetry: a requirement to perform an appropriate dose assessment. *Mutat Res Genet Toxicol Environ Mutagen.* Nov 2015;**793**:115–122. doi: 10.1016/j.mrgentox.2015.06.012
2. **Bender MA**, **Gooch PC**. Somatic chromosome aberrations induced by human whole-body irradiation: the "Recuplex" criticality accident. *Radiat Res.* Dec 1966;**29**(4):568–582.
3. **Dolphin GW**, **Bolton D**, **Humphreys DLO**, et al. Biological and physical dosimetry after a radiation accident. *Nature.* 1970;**227**(5254):165. doi: 10.1038/227165a0
4. **IAEA**. *Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies*. IAEA; 2011.
5. **Vaurijoux A**, **Gruel G**, **Pouzoulet F**, et al. Strategy for population triage based on dicentric analysis. *Radiat Res.* May 2009;**171**(5):541–548.
6. **Ramalho AT**, **Nascimento AC**, **Littlefield LG**, et al. Frequency of chromosomal aberrations in a subject accidentally exposed to 137Cs in the Goiania (Brazil) radiation accident: intercomparison among four laboratories. *Mutat Res.* Apr 1991;**252**(2):157–160.
7. **Voisin P**, **Benderitter M**, **Claraz M**, et al. The cytogenetic dosimetry of recent accidental overexposure. *Cell Mol Biol (Noisy -le-grand).* May 2001; **47**(3):557–564.
8. **Gregoire E**, **Hadjidekova V**, **Hristova R**, et al. Biological dosimetry assessments of a serious radiation accident in Bulgaria in 2011. *Radiat Prot Dosimetry*. Mar 3 2013;**155**(2):418–422. doi:nct032 [pii];10.1093/rpd/nct032 [doi]
9. **Lloyd DC**, **Prosser JS**, **Moquet JE**, **Malowany DJ**, *Doses in radiation accidents investigated by chromosome aberration analysis*. 1983. National Radiological Protection Board, Harwell (UK).
10. **Waselenko JK**, **Macvittie TJ**, **Blakely WF**, et al. Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. *Ann Intern Med*. Jun 15 2004; **140**(12):1037–1051. doi:140/12/1037 [pii]
11. **Roy L**, **Buard V**, **Delbos M**, et al. International intercomparison for criticality dosimetry: the case of biological dosimetry. *Radiat Prot Dosimetry.* 2004;**110**(1–4):471–476.
12. **Voisin P**, **Barquinero F**, **Blakely B**, et al. Towards a standardization of biological dosimetry by cytogenetics. *Cell Mol Biol (Noisy -le-grand).* Jul 2002;**48**(5):501–504.
13. **ISO**. *ISO 9001:2015 Quality management systems—requirements*. Geneva 2015.
14. **ISO/IEC**. *ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories*. Geneva 2017.
15. **ISO**. *ISO 19238:*2023 *Radiation protection—performance criteria for service laboratories performing biological dosimetry by cytogenetics.* 2023. ISO 19238:2023. 2023.
16. **ISO**. *ISO 21243—Radiation protection—performance criteria for laboratories performing cytogenetic triage for assessment of mass casualties in radiological or nuclear emergency.* Vol. ISO 21243. 2022. https://www.iso.org/standard/78139.html
17. **Norman A**, **Sasaki MS**, **Ottoman RE**, **Veomett RC**. Use of chromosome aberrations to estimate x-ray and gamma-ray dose to man. SAM-TR-67-112. *Tech Rep SAM-TR.* 5/23/1967 1967:1–18.
18. **Sasaki MS**, **Norman A.** Proliferation of human lymphocytes in culture. *Nature.* 1966;**210**:913–914.
19. **Grégoire E**, **Roy L**, **Buard V**, et al. Twenty years of FISH-based translocation analysis for retrospective ionizing radiation biodosimetry. *Int J Radiat Biol.* 2018;**94**(3):248–258. doi:10.1080/09553002.2018.1427903
20. **ISO**. *ISO 20046:2019 Radiological protection—performance criteria for laboratories using fluorescence in situ hybridization (FISH) translocation assay for assessment of exposure to ionizing radiation*. Geneva 2019.
21. **Sigurdson AJ**, **Ha M**, **Hauptmann M**, et al. International study of factors affecting human chromosome translocations. *Mutat Res.* Apr 30 2008; **652**(2):112–121.
22. **Nakano M**, **Kodama Y**, **Ohtaki K**, et al. Detection of stable chromosome aberrations by FISH in A-bomb survivors: comparison with previous solid Giemsa staining data on the same 230 individuals. *Int J Radiat Biol.* Sep 2001;**77**(9):971–977.
23. **Sevan'kaev AV**, **Lloyd DC**, **Edwards AA**, et al. A cytogenetic follow-up of some highly irradiated victims of the Chernobyl accident. *Radiat Prot Dosimetry.* 2005;**113**(2):152–161.

24. **Sevan'kaev AV**, **Khvostunov IK**, **Mikhailova GF**, et al. Novel data set for retrospective biodosimetry using both conventional and FISH chromosome analysis after high accidental overexposure. *Appl Radiat Isot.* May 2000; **52**(5):1149–1152.
25. **Camparoto ML**, **Ramalho AT**, **Natarajan AT**, et al. Translocation analysis by the FISH-painting method for retrospective dose reconstruction in individuals exposed to ionizing radiation 10 years after exposure. *Mutat Res.* Sep 29 2003;**530**(1–2):1–7.
26. **Natarajan AT**, **Santos SJ**, **Darroudi F**, et al. 137Cesium-induced chromosome aberrations analyzed by fluorescence in situ hybridization: eight years follow up of the Goiânia radiation accident victims. *Mutat Res.* May 25 1998;**400**(1–2):299–312. doi:10.1016/s0027-5107(98)00021-9
27. **Lindholm C**, **Edwards A.** Long-term persistence of translocations in stable lymphocytes from victims of a radiological accident. *Int J Radiat Biol.* Aug 2004;**80**(8):559–566.
28. **Lindholm C**, **Luomahaara S**, **Koivistoinen A**, et al. Comparison of dose-response curves for chromosomal aberrations established by chromosome painting and conventional analysis. *Int J Radiat Biol.* Jul 1998;**74**(1):27–34.
29. **Degteva MO**, **Vorobiova MI**, **Tolstykh EI**, et al. Development of an improved dose reconstruction system for the Techa River population affected by the operation of the Mayak Production Association. *Radiation Research.* 2006;**166**(1):255–270.
30. **ISO**. *ISO 17099:*2024 *Radiation protection—performance criteria for laboratories using the cytokinesis-blocked micronucleus assay (CBMN) in blood lymphocytes for biological dosimetry.* 2024. ISO 17099. 2024.
31. **da Cruz AD**, **McArthur AG**, **Silva CC**, et al. Human micronucleus counts are correlated with age, smoking, and cesium-137 dose in the Goiânia (Brazil) radiological accident. *Mutat Res.* 1994;**313**(1):57–68. doi: 10.1016/0165-1161(94)90033-7
32. **Fenech M**, **Perepetskaya G**, **Mikhalevich L.** A more comprehensive application of the micronucleus technique for biomonitoring of genetic damage rates in human populations—experiences from the Chernobyl catastrophe. *Environ Mol Mutagen.* 1997;**30**(2):112–118.
33. **Mikhalevich LS**, **De Zwart FA**, **Perepetskaya GA**, et al. Radiation effects in lymphocytes of children living in a Chernobyl contaminated region of Belarus [In Process Citation]. *Int J Radiat Biol.* Oct 2000;**76**(10): 1377–1385.
34. **Kanda R**, **Hayata I**, **Lloyd DC**. Easy biodosimetry for high-dose radiation exposures using drug-induced, prematurely condensed chromosomes. *Int J Radiat Biol.* Apr 1999;**75**(4):441–446.
35. **Pantelias GE**, **Maillie HD**. The use of peripheral blood mononuclear cell prematurely condensed chromosomes for biological dosimetry. *Radiat Res.* Jul 1984;**99**(1):140–150.
36. **Wojcik A**, **Gregoire E**, **Hayata I**, et al. Cytogenetic damage in lymphocytes for the purpose of dose reconstruction: a review of three recent radiation accidents. *Cytogenet Genome Res.* 2004;**104**(1–4):200–205.
37. **Hayata I**, **Kanda R**, **Minamihisamatsu M**, et al. Cytogenetical dose estimation for 3 severely exposed patients in the JCO criticality accident in Tokai-mura. *J Radiat Res (Tokyo).* Sep 2001;**42** Suppl:S149–S155.
38. **Yao B**, **Li Y**, **Liu G**, et al. Estimation of the biological dose received by five victims of a radiation accident using three different cytogenetic tools. *Mutat Res.* 2013;**751**(1):66–72. doi:10.1016/j.mrgentox.2012.11.005
39. **ISO**. *ISO 13304-1:*2020 *Radiation protection—minimum criteria for electron paramagnetic resonance (EPR) spectroscopy for retrospective dosimetry of ionizing radiation. Part 1: General principles.* 2020. ISO 13304-1. 2020.
40. **ISO**. *ISO 13304-2:*2020 *Radiation protection—minimum criteria for electron paramagnetic resonance (EPR) spectroscopy for retrospective dosimetry of ionizing radiation Part 2: ex vivo human tooth enamel dosimetry.* 2020. ISO 13304-2. 2020.
41. **Ghimire L**, **Waller E.** Electron paramagnetic resonance (EPR) biodosimetry with human teeth: a crucial technique for acute and chronic exposure assessment. *Health Physics.* 2024;**126**(5):322–338. doi:10.1097/hp.0000000000001822
42. **ICRU**. Retrospective assessment of exposures to ionising radiation. *J ICRU.* 2002;**2**. 1–136. ICRU Publication 68.
43. **ICRU**. Methods for initial-phase assessment of individual doses followIng acute exposure to ionizing radiation. *J ICRU.* Dec 01 2019;**19**:3–4. 1473-6691. Accessed Nov 20, 2024. doi:10.1177/1473669119895169
44. **Bougai ABA**, **Chumak V**, **Desrosiers M**, et al. *Use of Electron Paramagnetic Resonance Dosimetry With Tooth Enamel for Retrospective Dose Assessment*. International Atomic Energy Agency; 2002.
45. **ISO**. *ISO 21243:*2022 *Radiation protection—performance criteria for laboratories performing cytogenetic triage for assessment of mass casualties in radiological or nuclear emergencies—general principles and application to dicentric assay.* 2022. ISO 21243:2022. 2022.
46. **Kulka U**, **Wojcik A**, **Di Giorgio M**, et al. Biodosimetry and biodosimetry networks for managing radiation emergency. *Radiat Prot Dosim.* 2018; **182**(1):128–138. doi:10.1093/RPD/NCY137
47. **Blakely WF**, **Carr Z**, **Chu MC**, et al. WHO 1st consultation on the development of a global biodosimetry laboratories network for radiation emergencies (BioDoseNet). *Radiat Res.* Jan 2009;**171**(1):127–139.
48. **Wilkins RC**, **Lloyd DC**, **Maznyk NA**, **Carr Z.** The international biodosimetry capacity, capabilities, needs and challenges: the 3rd WHO BioDoseNet survey results. *Environ Adv.* 2022;**8**:1694–1703. doi:10.1016/j.envadv.2022.100202
49. **Endesfelder D**, **Oestreicher U**, **Bucher M**, et al. RENEB inter-laboratory comparison 2021: the dicentric chromosome assay. *Radiat Res.* 2023; **199**(6):556–570, 15.
50. **Miller SM**, **Ferrarotto CL**, **Vlahovich S**, et al. Canadian Cytogenetic Emergency Network (CEN) for biological dosimetry following radiological/nuclear accidents. *Int J Radiat Biol.* 2007;**83**(7):471–477.
51. **Wilkins RC**, **Beaton-Green LA**, **Lachapelle S**, et al. Evaluation of the annual Canadian biodosimetry network intercomparisons. *Int J Radiat Biol.* 2015;**91**(5):443–451. doi:10.3109/09553002.2015.1012305
52. **García O**, **Di Giorgio M**, **Radl A**, et al. The Latin American Biological Dosimetry Network (LBDNet). *Radiat Prot Dosimetry.* 2016;**171**(1):64–69. doi:10.1093/rpd/ncw209
53. **Kurihara O**, **Ha WH**, **Qinjian C**, **Jang S**. ARADOS: Asian network for radiation dosimetry. *Radiat Meas*. 2020;**135**. doi:10.1016/j.radmeas.2020.106336
54. **Vijayalakshmi J**, **Chaurasia RK**, **Srinivas KS**, et al. Establishment of ex vivo calibration curve for X-ray induced "dicentric + ring" and micronuclei in human peripheral lymphocytes for biodosimetry during radiological emergencies, and validation with dose blinded samples. *Heliyon.* 2023;**9**(6): e17068. doi:10.1016/j.heliyon.2023.e17068
55. **Waldner L**, **Bernhardsson C**, **Woda C**, et al. The 2019–2020 EURADOS WG10 and RENEB field test of retrospective dosimetry methods in a small-scale incident involving ionizing radiation. *Radiat Res*. Mar 1 2021;**195**(3): 253–264. doi:10.1667/rade-20-00243.1
56. **Kulka U**, **Wojcik A.** Special issue: networking in biological and EPR/OSL dosimetry: the European RENEB platform for emergency preparedness and research. *Int J Radiat Biol.* 2017;**93**(1):1. doi:10.1080/09553002.2016.1235805
57. **Port M**, **Oestreicher U.** Editorial for the Special Issue on RENEB ILC 2021. *Radiat Res.* 2023;**199**(6):533–534, 2.
58. **Gregoire E**, **Barquinero JF**, **Gruel G**, et al. RENEB Inter-Laboratory comparison 2017: limits and pitfalls of ILCs. *Int J Radiat Biol.* 2021;**97**(7): 888–905. doi:10.1080/09553002.2021.1928782
59. **Trompier F**, **Burbidge C**, **Bassinet C**, et al. Overview of physical dosimetry methods for triage application integrated in the new European network RENEB. *Int J Radiat Biol.* 2017;**93**(1):65–74. doi:10.1080/09553002.2016.1221545
60. **Ainsbury E**, **Badie C**, **Barnard S**, et al. Integration of new biological and physical retrospective dosimetry methods into EU emergency response plans—joint RENEB and EURADOS inter-laboratory comparisons. *Int J Radiat Biol.* Jan 2017;**93**(1):99–109. doi:10.1080/09553002.2016.1206233 [doi]
61. **Wilkins RC**, **Beaton-Green LA**, **Lachapelle S**, et al. Evaluation of the annual Canadian biodosimetry network intercomparisons. *Int J Radiat Biol.* May 2015;**91**(5):443–451. doi:10.3109/09553002.2015.1012305
62. **Fattibene P**, **Wieser A**, **Adolfsson E**, et al. The 4th international comparison on EPR dosimetry with tooth enamel: Part 1: Report on the results. *Radiat Meas.* Sep 01 2011;**46**(9):765–771. doi:https://doi.org/10.1016/j.radmeas.2011.05.001
63. **Port M**, **Barquinero JF**, **Endesfelder D**, et al. RENEB inter-laboratory comparison 2021: inter-assay comparison of eight dosimetry assays. *Radiat Res*. Jun 1 2023;**199**(6):535–555. doi:10.1667/rade-22-00207.1

https://doi.org/10.1017/dmp.2025.10095 Published online by Cambridge University Press

64. **Sedelnikova OA**, **Pilch DR**, **Redon C**, **Bonner WM**. Histone H2AX in DNA damage and repair. *Cancer Biol Ther*. May 2003;**2**(3):233–235.
65. **Andrievski A**, **Wilkins RC**. The response of gamma-H2AX in human lymphocytes and lymphocytes subsets measured in whole blood cultures. *Int J Radiat Biol*. Apr 2009;**85**(4):369–376. doi:10.1080/09553000902781147
66. **Rothkamm K**, **Horn S.** Gamma-H2AX as protein biomarker for radiation exposure. *Ann Ist Super Sanita*. 2009;**45**(3):265–271.
67. **Abend M**, **Ostheim P**, **Port M.** Radiation-induced gene expression changes used for biodosimetry and clinical outcome prediction: challenges and promises. *Cytogenet Genome Res.* 2023;**163**(3–4):223–230. doi:10.1159/000530947
68. **Brzoska K**, **Kruszewski M.** Toward the development of transcriptional biodosimetry for the identification of irradiated individuals and assessment of absorbed radiation dose. *Radiat Environ Biophys.* Aug 2015;**54**(3):353–363. doi:10.1007/s00411-015-0603-8
69. **Cruz-Garcia L**, **O'Brien G**, **Donovan E**, et al. Influence of confounding factors on radiation dose estimation using in vivo validated transcriptional biomarkers. *Health Phys.* Jul 2018;**115**(1):90–101. doi:10.1097/HP.0000000000000844
70. **Brzóska K**, **Abend M**, **O'Brien G**, et al. Calibration curve for radiation dose estimation using FDXR gene expression biodosimetry—premises and pitfalls. *Int J Radiat Biol.* Aug 02 2024;**100**(8):1202–1212. doi:10.1080/09553002.2024.2373751
71. **Ostheim P**, **Alemu SW**, **Tichý A**, et al. Examining potential confounding factors in gene expression analysis of human saliva and identifying potential housekeeping genes. Sci Rep. *Feb* **10** 2022;12(1):2312. doi:10.1038/s41598-022-05670-5
72. **Ostheim P**, **Tichý A**, **Badie C**, et al. Applicability of gene expression in saliva as an alternative to blood for biodosimetry and prediction of radiation-induced health effects. *Radiat Res.* May 1 2024;**201**(5):523–534. doi:10.1667/rade-23-00176.1
73. **Furukawa A.** The project of another low-cost metaphase finder (second report-application of artificial intelligence). *Radiat Prot Dosimetry*. Dec 31 2019;**186**(1):37–41. doi:10.1093/rpd/ncz012
74. **Weber J**, **Scheid W**, **Traut H.** Time-saving in biological dosimetry by using the automatic metaphase finder Metafer2. *Mutat Res.* Aug 1992;**272**(1):31–34. doi: 10.1016/0165-1161(92)90006-8
75. **Romm H**, **Ainsbury E**, **Barnard S**, et al. Validation of semi-automatic scoring of dicentric chromosomes after simulation of three different irradiation scenarios. *Health Phys*. Jun 2014;**106**(6):764–771. doi: 10.1097/HP.0000000000000077
76. **Lee Y**, **Kim SH**, **Lee YH**, et al. Application of a semi-automated dicentric scoring system in triage and monitoring occupational radiation exposure. *Frontiers in Public Health*. 2022;**10**. doi:10.3389/fpubh.2022.1002501
77. **Schunck C**, **Johannes T**, **Varga D**, et al. New developments in automated cytogenetic imaging: unattended scoring of dicentric chromosomes, micronuclei, single cell gel electrophoresis, and fluorescence signals. *Cytogenet Genome Res.* 2004;**104**(1–4):383–389. doi:10.1159/000077520
78. **Liu J**, **Li Y**, **Wilkins R**, et al. Accurate cytogenetic biodosimetry through automated dicentric chromosome curation and metaphase cell selection. *F1000Res.* 2017;**6**:1396. doi:10.12688/f1000research.12226.1
79. **Rogan PK**, **Li Y**, **Wilkins R**, et al. Radiation dose estimation by automated cytogenetic biodosimetry. *Radiat Prot Dosimetry*. Jul 13 2016;**172**(1–3):207–217. doi:10.1093/rpd/ncw161
80. **Kim K**, **Kim KS**, **Jang WI**, et al. Deep neural network-based automatic dicentric chromosome detection using a model pretrained on common objects. *Diagnostics (Basel)*. Oct 12 2023;**13**(20). doi:10.3390/diagnostics13203191
81. **Shen X**, **Ma T**, **Li C**, et al. High-precision automatic identification method for dicentric chromosome images using two-stage convolutional neural network. *Sci Rep*. Feb 6 2023;**13**(1):2124. doi:10.1038/s41598-023-28456-9
82. **Jeong SK**, **Oh SJ**, **Kim SH**, et al. Dicentric chromosome assay using a deep learning-based automated system. *Sci Rep*. Dec 21 2022;**12**(1):22097. doi:10.1038/s41598-022-25856-1
83. **Schunck C**, **Lorch T**, **Kowalski R**, et al. A standardized and automated CBMN assay for biological dosimetry: the CytoRADx system. *Radiat Prot Dosimetry*. Sep 18 2023;**199**(14):1516–1519. doi:10.1093/rpd/ncad027
84. **Rodrigues MA**, **Beaton-Green LA**, **Kutzner BC**, **Wilkins RC**. Automated analysis of the cytokinesis-block micronucleus assay for radiation biodosimetry using imaging flow cytometry. *Radiat Environ Biophys*. May 2014;**53**(2):273–282. doi:10.1007/s00411-014-0525-x
85. **Thierens H**, **Vral A**, **Vandevoorde C**, et al. Is a semi-automated approach indicated in the application of the automated micronucleus assay for triage purposes? *Radiat Prot Dosimetry*. Jun 2014;**159**(1–4):87–94. doi:10.1093/rpd/ncu130
86. **Seager AL**, **Shah UK**, **Brusehafer K**, et al. Recommendations, evaluation and validation of a semi-automated, fluorescent-based scoring protocol for micronucleus testing in human cells. *Mutagenesis*. May 2014;**29**(3):155–164. doi:10.1093/mutage/geu008
87. **Flood AB**, **Sidabras JW**, **Swarts SG**, et al. Benefits and challenges of in vivo EPR nail biodosimetry in a second tier of medical triage in response to a large radiation event. *Radiat Prot Dosimetry*. Sep 18 2023;**199**(14):1539–1550. doi:10.1093/rpd/ncad022
88. **Flood AB**, **Williams BB**, **Schreiber W**, et al. Advances in in vivo EPR Tooth BIOdosimetry: meeting the targets for initial triage following a large-scale radiation event. *Radiat Prot Dosimetry*. Jul 15 2016. doi:10.1093/rpd/ncw165
89. **Singh VK**, **Swartz HM**, **Seed TM**. Radiation medical countermeasures and use of EPR biodosimetry to facilitate effectiveness of applied clinical procedures. *Appl Magn Reson.* 2022;**53**(1):289–303. doi:10.1007/s00723-021-01444-w
90. **Romanyukha A**, **Trompier F**, **Reyes RA**. Q-band electron paramagnetic resonance dosimetry in tooth enamel: biopsy procedure and determination of dose detection limit. *Radiat Environ Biophys*. 2014;**53**(2):305–310. doi:10.1007/s00411-013-0511-8
91. **Fattibene P**, **Trompier F**, **Bassinet C**, et al. Reflections on the future developments of research in retrospective physical dosimetry. *Physics Open*. 2023;14. doi:10.1016/j.physo.2022.100132
92. **Choudhury T.** Deep Learning Applications and Interpretation in Signal Processing and Magnetic Resonance. Doctoral. University of Southampton; 2024. https://eprints.soton.ac.uk/493825/

https://doi.org/10.1017/dmp.2025.10095 Published online by Cambridge University Press